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Tradescantia Micronucleus Bioassay and Pollen Tube Chromatid Aberration Test for *in Situ* Monitoring and Mutagen Screening

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The meiotic pollen mother cells (PMC) of Tradescantia (spiderwort) are highly synchronized in their prophase I and tetrad stages. Chromosomes of this stage are sensitive to physical or chemical mutagens. Thus high frequency of acentric fragments or sticky chromosomes can be induced with very low level of mutagens. These induced chromosome aberrations become micronuclei (MCN) in the synchronized tetrads and they can be easily identified and scored. Based upon these features, the Tradescantia micronucleus bioassay was established. This bioassay involves the exposure of PMC in the young inflorescences of the plant cuttings to gaseous agents through diffusion, to liquid agent through absorption and dialysis from the stem to flower buds, or to radiation. The exposed samples are fixed in aceto-alcohol (1:3) and prepared into microslides by using the aceto-carmine squash method. Frequencies of MCN in a large population of synchronized tetrads are the indications of genetic damage caused by the agents.

Mature pollen grains of Tradescantia are free cells which can be cultured in lactose-agar medium. The generative cells in the cultured pollen tubes can carry out mitosis similar to the *in vivo* condition. The G_2 interphase chromosomes of pollen mitosis are highly sensitive to gaseous or liquid chemicals and radiation. Treatments can be applied to these mitotic generative cells of the mature pollen or to the mitotic generative nuclei of the developing pollen tube. The mitotic chromosomes of the generative cells are allowed to proceed through mitosis in the culture medium and slides are prepared for metaphase figures. Frequencies of various types of chromatid aberrations can be scored and used as the indices of genetic damage.

Introduction

Tradescantia, commonly called spiderwort, is a herbaceous plant which has almost world-wide adaptation and can grow around the year in the field of subtropical regions of the world or in greenhouses everywhere. Its relative small plant size (most species are less than 50 cm in height) and six pairs of relatively large chromosomes in its somatic cells made it a favorable experimental material for cytogenetic studies. Plants can sustain their sexual reproductive state and give rise to buds and flowers by the help of supplemented light during the short-day season.

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Tradescantia paludosa clone #03, a prolific, robust and multi-branched species became a popular cytogenetic experimental material since the late 1930s (*1–5*). Extensive studies of the direct and indirect effects of radiation on mitotic chromosomes of microspores of Tradescantia laid the foundations of a number of principles on radiation induction of chromosome breakage, the mechanism of chromosome aberration and the interpretation of the aberrant chromosome and chromatid configuration (*3, 4, 6*). The root tip chromosomes of Tradescantia were used for studies of chemical mutagen and radiation induced chromosome damage.

Tradescantia pollen tube chromosomes of the mitotic generative cells were used for the study of ionizing radiation and ultraviolet light induced chromatid aberrations since late 1940s (*7-11*). When

mature pollen grains were cultured on a simple lactose-agar medium, radiation (*5, 7, 8, 10*) or chemical mutagen (*7, 9, 12, 13*) treatment to the pollen or the mitotic generative nucleus of the newly germinated pollen tube can induce chromatid aberrations (Fig. 1)

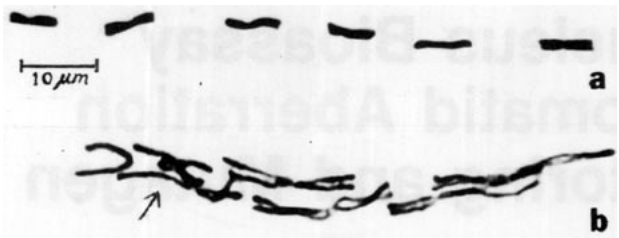

FIGURE. 1 Tradescantia pollen tube chromosomes: (a) normal c-metaphase, (b) chromatid aberration, asymmetrical interchange (arrow).

Meiotic chromosomes of pollen mother cells (PMC) have long been known to be more sensitive than mitotic chromosomes to radiation (*3, 4, 6, 14*) but they were rarely used for studies on the induced chromosome aberrations because the aberrations were hard to be analyzed and scored to compile quantitative data. The major problem was due to the looseness of the metaphase I chromosomes and their unclear image in the meiotic PMC preparations. However, if the treatment is applied to the early prophase I stage and allow the treated meiotic chromosome to go through a proper recovery time (*15*), the acentric fragments or the sticky chromosome complex would become micronuclei (MCN) in tetrad stage of meiosis (Fig. 2). This was the basis for establishing the Tradescantia micronucleus (Trad-MCN) bioassay (*16, 17*). This bioassay was first utilized in the study of mutagenic effects of

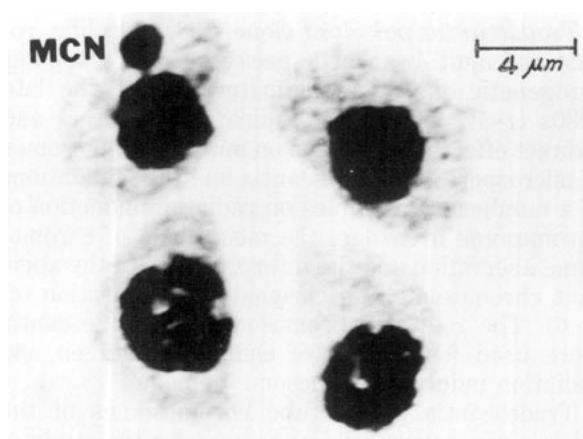

FIGURE. 2 Tradescantia meiotic pollen mother cell, tetrad containing micronucleus (MCN).

1,2-dibromoethane (*16*) and followed by x-rays and known mutagens (*17*). The dose-response curves were established by 1,2-dibromoethane and x-ray treatment (*14*). The linear regression dose-response curve of x-rays is shown in Figure 3.

The present paper intends to give a general review on the use of Tradescantia pollen systems in the detection of the mutagenic effects of radiation and chemical agents. General procedures of Trad-MCN bioassay and pollen tube chromatid aberration test will be described, and their favorable features and limitations will be discussed.

Tradescantia Micronucleus Bioassay

Experimental Procedure

A detailed account of this bioassay was given elsewhere (*18*). A general outline of the procedure is given here with special remarks on the favorable features and limitations of this bioassay.

Selection of Buds for Treatment. An inflorescence of *Tradescantia paludosa* clone #03 may contain as many as 20 buds in which the largest bud in the series is usually ready to bloom within 24-48 hr. This kind of inflorescence is in the ideal stage for treatment because most likely one of the younger

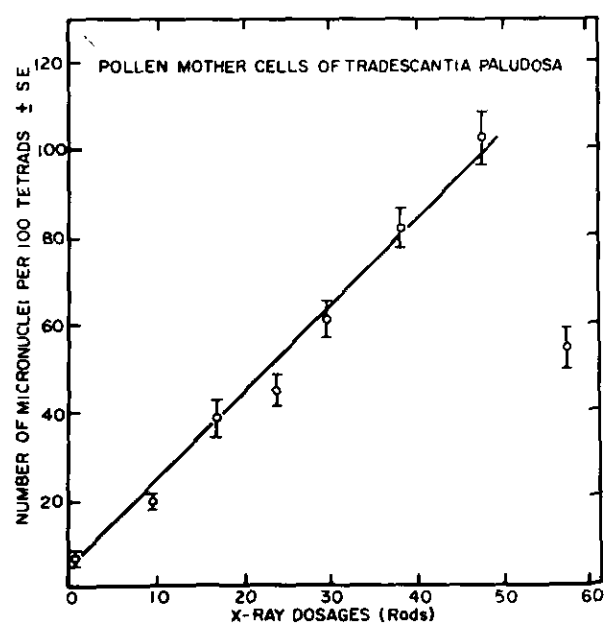

FIGURE. 3 Dose-response curve established by increasing doses of x-rays.

buds is undergoing early meiotic prophase I division which is sensitive to mutagens. The selected inflorescences are excised from the plant (about 5-8 cm in length) and maintained in water cups in groups of 15 to 20.

Treatment Procedures. Gaseous agents can be applied in an enclosed chamber with known concentration of gas and the rate of flow. The PMC of the plant receives the treatment through diffusion of the gas into the buds (*19*). The plant cuttings in the chamber should be illuminated during the treatment period thus the normal life processes can be maintained. The duration of treatment is usually 6 hr but occasionally may be extended up to 24 hr when low level of gas is used.

Liquid treatment can be applied by adding a known quantity of agent in water. The water solution of the agent is readily absorbed through the stem, peduncle and pedicel up to the filament of the stamen. Water-insoluble agents are usually first dissolved in dimethyl sulfoxide (DMSO) or ethanol. Treatment duration is usually 6 to 24 hr.

In situ monitoring for gaseous pollutants in the air is conducted by exposing the plant cuttings to the atmosphere of the selected sites for the durations raging from 2 to 6 hr. This can also be done by growing the plants on site and sampling the inflorescences for cytological observations from time to time. For water pollutants from runoffs of industrial sites or power plants, monitoring can be conducted either by growing plants in nearby fields or taking water samples to be tested in the laboratories.

Among physical agents tested by this bioassay, ultraviolet light, x-rays, and γ-rays were proven to be effective in damaging chromosomes and resulting in increase of MCN frequencies. Radiation treatments are administered by exposing the plant cuttings to the physical agents at a given distance and duration.

Fixation of Samples and Preparation of Microslides. The inflorescences are fixed in aceto-alcohol (1:3) after a 24 or 30 hr recovery time. After 24 hr of fixation, the samples are stored in 70% ethanol. Since the MCN to be scored are in the well-synchronized early tetrad stage, therefore, only the buds which contain early stage tetrads (four cells encased in an envelope) are selected for preparation of microslides. The aceto-carmine squash technique is used and described here step-by-step in Figure 4. The selection of proper buds is done on the basis of size and relative position among the series of buds in a given inflorescence. A pair of dissecting needles are used to remove the glumes and expose the anthers. The meiotic PMC are released from anthers by crushing the anthers on the slide. A drop

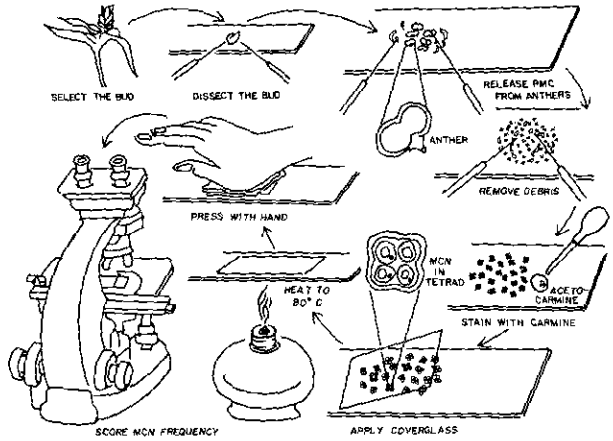

FIGURE. 4 Step-by-step illustration of the aceto-carmine squash technique for preparation of tetrad slides.

of aceto-carmine stain is applied over the content of the crushed anthers and allow 2-5 min to stain the nuclei of the cells. When the correct stage of PMC (tetrad stage) is secured by the help of a magnifying lens or a low power dissecting microscope, the debris from the broken anther wall or stamen hair are carefully removed with the dissecting needles before application of the cover glass. The slide with the cell contents under the cover glass is heated (below boiling) repeatedly over an alcohol flame to improve the staining quality. Gentle pressure is applied over the coverglass with the palm of hand which is protected from excessive heat by several layers of absorbent paper. This temporary slide is ready to be scored under 400 × magnification.

Scoring of MCN Frequency. Generally each experimental group is composed of 15 to 20 plant cuttings from which 5 to 10 microslides can be made. Although each well prepared microslide contains thousands of tetrads, only about 300 tetrads are randomly sampled in the scoring process. Each slide is considered to be a sample population. Variance and standard deviation are derived from 5 to 10 slides (1,500 to 3,000 tetrad per experimental group). One tetrad may contain 1 to 5 or occasionally up to 16 MCN, thus tetrads containing different number of MCN are scored under different columns of the score sheet. The total number of MCN in a given slide is tallied and divided by the number of tetrads scored. The fraction is the expression of number of MCN per tetrad, or the percentage is the expression of number of MCN per 100 tetrads.

Experimental Results

Among the chemical and physical agents tested by this bioassay and published in the literature are

1,2-dibromoethane (*16*), sodium azide, EMS (*17*), cyclohexylamine, maleic hydrazide (*20*), X-rays (*14, 17*) and ultraviolet light (*20*). Fourteen other chemicals in the forms of gas and liquid were tested and reported at the 11th Annual Meeting of the Environmental Mutagen Society (*21*). A sample of these experimental data from the latest tests on benzo(a)pyrene (liquid), hydrazoic acid (gas) and *in situ* monitoring (truck stop) are shown in Table 1.

Favorable Features of the TRAD-MCN Bioassay

Trad-MCN bioassay is a simple, rapid test which can yield reliable results in 24-48 hr. By treating the mutagen-sensitive and highly synchronized early prophase I PMC and scoring MCN in the fully synchronized tetrads, this bioassay can detect the effect of 5 rad of x-rays, 5 ppm of 1,2-dibromoethane, or 1 ppm of SO_2. Its versatility was demonstrated by the test results on gaseous (*16, 17, 21, 22*), liquid (*17, 21*) chemical agents and radiation (*14, 17, 20*) as well as *in situ* monitoring (*21*).

Tradescantia plants are clones of the same genetic make-up and propagated by asexual means, thus the genetic homogeneiety can be maintained. Pollen mother cells are germ cells of higher plants. The magnitude of chromosome damage in PMC can be used to estimate the genetic damage which is transmitted to next generation. The plant cuttings used in the experiments can be considered as *in vivo* as well as *in vitro* experimental materials. Enzymatic activation is often unnecessary because the agents for treatment can be metabolized in plant system without animal activators. Treated sample (fixed inflorescences) can be stored away in large quantities for cytological observation and analysis at the later time. The slides which bear tetrads and MCN frequency records can be made into permanent preparation for future study and confirmation. Average sizes of tetrads (18–22 μm) and MCN (0.5-3 μm) are relatively large, therefore, they can be easily identified and scored under 400 × magnification.

Limitations of the TRAD-MCN Bioassay

Since the chromosome damage in the form of MCN is used as the end point, results of this test serve only as a relative degree of damage. Translocations, inversions and other types of chromosome, chromatid rearrangements and exchanges are not revealed as MCN in tetrads. No carcinogenic effect of the agent can be detected by this bioassay nor the relative carcinogenicity of the agent can be easily extrapolated from MCN frequencies. The metabolic pathway of a given mutagen or promutagen in Tradescantia could be quite different from that in a mammalian system. Therefore, a positive response in Trad-MCN test may not necessarily be true in mammalian or human systems. This bioassay can not be used to determine the cumulative effect of mutagens because this test utilizes only a portion of the meiotic cycle in each bud of the inflorescence unless the agent is concentrated and accumulated in the plant body when the whole plants are used. Due to the high sensitivity of the

Table 1. Results of Trad-MCN bioassay on gaseous and liquid agents and in situ monitoring of air pollutants.

Exptl. group	Code number	Treatment dosage	Duration of exposure, hr	MCN/100 tetrads	S.E.	Signif. $P > 0.01$
BaP[a]–2	BC[b]	0	–	5.10	1.17	
	C_d[c]	0	6	7.97	0.62	
	T–1	0.05 *mM*	6	15.50	1.52	+
	T–2	0.10 *mM*	6	23.13	3.78	+
HN_3–1[d]	C	0	–	5.20	0.58	
	T–1	136 ppm	6	21.20	3.66	+
	T–2	272 ppm	6	14.30	0.90	+
WTS–1[e]	HC[f]	–	–	5.12	0.69	
	FC[g]	–	–	5.62	0.49	
	T–1	–	2.5	10.54	1.05	+
	T–2	–	5.0	10.84	0.80	+

[a] BaP = benzo(a)pyrene.
[b] BC = baseline control.
[c] Cd = DMSO control.
[d] HN_3 = hydrazoic acid.
[e] WTS = Woodhull Truck Stop.
[f] HC = home control.
[g] FC = field control.

system, the background MCN frequencies often show day-to-day variation. The growing conditions of plants, such as purity of the air, consistency of the growing medium, fertilizer used and light exposure, have to be kept constant.

Tradescantia Pollen Tube Chromatid Aberration Test

Experimental Procedure

The technical details on culturing pollen tubes, treatment procedures and microslide preparation were elaborated in several earlier publications (*1, 2, 23*). X-rays (*7, 8*), ultraviolet light (*5, 10*), sulfur dioxide and organic gases (*9, 11, 13*), and hydroxyurea (*12*) treatments were applied to this system and positive responses were obtained from relatively low doses of these mutagens. A general outline of this test is given below.

Pollen grains of Tradescantia are collected from fully opened flowers in the morning and desiccated in the dark for at least 4 hr. Dry pollen grains can be sown on semisolidified lactose-agar medium at the temperature around 38°C with the help of a camel hair brush. Treatment with radiation or gaseous agents can be applied to dry pollen before sowing on the medium or applied to newly germinated pollen tubes while the G_2 interphase chromosomes are most sensitive to mutagens. The treated and control pollen tube cultures grown on the medium coated slides are immediately incubated in the moist chambers at 22°C in the dark for 16 hr. With the help of colchicine, which is a part of the culture medium, some of the mitotic generative cells are arrested at metaphase at the end of the 16 hr incubation period. Aceto-alcohol (1:3) or Gate's fluid is usually used to fix the culture. Hot acid (HCl, 60°C) hydrolysis and hot water (80°C) treatment help to delaminate the solidified agar medium from the slide surface. This is followed by cold water flushing in order to remove the medium from the slide. Feulgen reaction is used to stain the chromosomes after the removal of the medium. Permanent slides are prepared by passing the slide through a series of dehydration ethanol and mounting in Euparal.

Favorable Features and Limitations

This test is an extreme sensitive bioassay for gaseous agents if treatment is applied to the early stage of pollen germination. This feature could be counted as a favorable quality as far as the mutagenic response is concerned but at the same time, this high sensitivity could also be viewed as limiting factor for its general application where the pollutants level is too high for the test. This response was demonstrated in the studies of SO_2 where 0.1 ppm concentration could increase the aberration frequency to 2–3 times of the control level (*9*), and 5 ppm and higher often inhibit the mitotic activity and the growth of the tube (*11*).

Since the chromosomes of generative cells in the mature pollen of Tradescantia is fully doubled, therefore, aberrations induced at pollen or pollen tube stage would be chromatid types. Scoring of chromatid aberration under 1000 × magnification is a painstaking and time consuming task.

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